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Gene transfer vector for the diagnosis and the therapy of malign tumours

The invention relates to a new gene transfer vector and its use, in particular for the treatment of chemo-resistant tumour cells.

It is known that about 50% of all tumours cannot be treated as they are "multi-drug resistant". For example, breast cancer cells can be either primarily resistant against chemotherapy or can develop this resistance in a later phase after initially successful therapy (secondary therapy resistance).
Such a resistant phenotype can result from the over-expression of a transporter protein. This so-called *P-Glycoprotein* forms a kind of pump for example for the above mentioned chemotherapy as a trans-membrane protein, by which these therapeutics are transported back into the extra-cellular area. The P-Glycoprotein is coded by the MDR-1 ("Multi-drug resistance") gene, which is regulated on a transcriptional level by the YB-1 binding protein, the latter binding on the "Y box" within the DNA sequence of the MDR-1 gene (Van Veen and Konings et al, 1997, Sem Cancer Biol, 8, 183-191).

The YB-1 promoter controls the expression of the YB-1 protein, which is a member of the family of the "Y box" binding proteins. These Y box factors belong to a highly conserved class of

proteins which play a role in the regulation of *transcription* and *translation*. The proteins bind to a sequence within the DNA of a target gene (the so-called Y box sequence), by which the expression of this gene results (Bargou et al, 1997, Nat Med, 3, 447-450).

YB-1 is strongly expressed in the course of cell proliferation and can be induced by genotoxic substances, e.g. chemo-therapeutics, UV light and ionising radiation (Koike et al, 1997, Febs Lett, 417, 390-394). In addition, it has been established that the expression of YB-1 is considerably increased in proliferating cells such as embryonal and regenerating tissues, whereas this condition is reversed in tissue differentiation (Grant and Deeley, 1993, Mol Cell Biol, 12, 4186-4196, Spitkovsky et al, 1992, Nucleic Acido Res, 20, 797-803).
Our own studies have shown that the over-expression of the MDR-1 gene in breast cancer cells and the intrinsic multi-drug resistance connected therewith are connected with the activity and localisation of the YB-1 protein (Bargou et al, 1997, Nat Med, 3, 447-450).

In cases of chemo-resistance, it is therefore necessary to find an alternative to the use of chemo-therapeutics.

It is known that gene therapy is used for the treatment of acquired and inherited diseases, with it being a question of the transfer of a therapeutic gene, for example a tumour suppressor gene. Various vector systems are available in this context, in order to achieve the highest possible share of cells of the target tissue in the gene transfer. In this, viral vectors have proven to be most suited up to now. Adenoviral vectors are being used increasingly more often as they can infect a plurality of tumour tissues with great effectivity. Each of these vectors contains a specific expression cassette (EC), which enters the target cell through the adenoviral infection. This expression cassette comprises a promoter and a therapeutic gene, with the promoter ensuring the expression of the gene in the target cells. Promoters frequently used are, for example, SV40, RSV and CMV (Sandig et al, 1997, Nat med, 3, 313-319).

The fact that adenoviruses can infect a number of types of tissue is, on the one hand, a benefit of this gene transfer system. Customary adenoviral strategies, on the other hand, are insufficient in certain diseases/therapies. It is therefore necessary to develop therapies in which the gene expression is only restricted to certain cells. This can be achieved by *tissue-specific promoters*. The use, for example, of tumour-specific promoters creates the possibility of only expressing the therapeutic gene in the tumour tissue and not in the adjoining,

likewise infected standard tissue (Robbins et al, 1998, Trends Biotechnol, 16, 35-40). In this way, the target precision of the gene transfer is increased, by which it becomes possible also to use therapeutic genes which are damaging for normal cells.

The invention was thus based on the task of developing a vector with an expression cassette containing a tumour-specific promoter which only expresses a relevant gene in chemo-resistant tumour cells. This gene therapy vector is thus to be used in tumour cells which are already chemo-resistant and thus no longer react to a conventional chemotherapy.

On the one hand, the cassette is to be constructed in such a way that it can be cloned into various gene transfer vectors, for example adenoviral vectors. On the other hand, it is simultaneously to be possible to clone the varied therapeutic genes into this cassette "downstream" from the promoter without great technical efforts.

The task is solved according to the patent claims by a vector possessing the following components: the YB-1 promoter, a transgene and two "multiple cloning sites" (MCS). In this, the tumour-specific YB-1 promoter is to bring a transgene to expression in chemo-resistant tumour cells by adenoviral gene transfer. This transgene can be a therapeutically relevant gene

such as an apoptosis-inducing gene, through which a demise of the tumour cells is initiated. But it can also be a "prodrug converting enzyme", which converts a certain molecule added from the outside ("prodrug") into a pharmacologically active agent, which then has its therapeutic effect on the tumour cells. In addition, two different therapeutically relevant genes can be placed under the control of the YB-1 promoter in a so-called double-gene transfer.

In this, the YB-1 promoter is cloned into a correspondingly adapted MCS of a vector. The latter is characterised in that it contains a number of selected enzyme restriction sites which permit cloning a new therapeutic gene into the expression cassette "downstream" of the promoter without changes having to be made to the remaining vector or to the YB-1 promoter already to be found in the vector.

The new gene transfer vector can be used for the treatment of tumours. Preferably, it is suitable for the treatment of chemo-resistant tumours. A further possibility of application is in diagnosis (micro-localisation of tumours).

EXAMPLE OF EMBODIMENT 1

To start with, the corresponding therapeutic gene is cloned behind the YB-1 promoter (nucleotide 259-294 of the MCS of the

pCR2.1 vector of Invitrogen and nucleotide 453-2150 of the YB-1 promoter sequence, gene bank Acc.# X96666). These two elements represent the expression cassette (see Fig. 1). In this, the YB-1 promoter is cloned into an MSC of a vector adapted specifically for this purpose in such a way that various therapeutic genes can be put under the control of the promoter without the MCS having to be adapted again. This is a MCS containing a group of specifically selected enzyme restriction sites. These restriction sites are to permit a quick and uncomplicated exchange of the therapeutic genes into the expression cassette "downstream" of the promoter. Additional changes to the remaining vector and to the YB-1 promoter already existing are avoided in this way. Thus, there results a kind of "box-of-bricks" system in which the therapeutic genes can be replaced with a low amount of effort (see Fig. 2).

The expression cassette containing the YB-1 promoter and the therapeutic gene is then cloned into a so-called transfer plasmide (TP). This plasmide contains a part of the adenoviral genome. For this step, the enzyme restriction sites of the MCS surrounding the EC and also existent in the transfer plasmide are used. The TP is then transformed into bacteria (BJ cells) with the "helper plasmide" (HP). The helper plasmide possesses the entire adenoviral genome with the exception of the E1 and E3 region, with the E1 deletion making the virus replication-

deficient. As the adenoviral genome sections surrounding the EC in the TP have a homology with certain sections of the genome of the HP, a recombinant adenoviral plasmide containing the expression cassette (see Fig. 3) is generated by homologous recombination in the BJ cells. By gene transfer techniques such as calcium phosphate precipitation or liposomes, this recombinant adenoviral plasmide is then inserted into a production cell line (293; human, embryonal renal cell line), in order to lead to production of replication-deficient viruses. The latter contain the therapeutic gene under the control of the tumour-specific YB-1 promoter. After this, tumours of chemo-resistant cell lines are established in various mouse strains (SCID and nude mice) (e.g. of epithelial origin), which are then infected with the recombinant virus. The measurement of the transgene expression by e.g. ELISA and immuno-histochemical techniques and its effect on the tumour are then analysed with regard to a possible therapeutic approach.

EXAMPLE OF EMBODIMENT 2

The invention was checked in vivo for the proliferation-specific activity of the YB-1 promoter in an animal model. It is known that adenoviral vectors drive hepatocytes into proliferation on the third day after vector application. For this reason, two adenoviral vectors were compared in SCID mice with human alpha 1-

antitrypsin (hAAT), which were only distinguished by the promoter (AdYB-1.hAAT or AdRSV.hAAT) in a liver gene transfer model.

The constitutive promoter led to a continuous increase of the serum content of hAAT (Fig. 4A). In contract, the Ad vector with the YB-1 promoter led to a temporarily very high expression with a maximum serum content of hAAT on the third day (Fig. 4B).

1.0×10^9 pfu AdRSV.hAAT (A) or AdYB-1.hAAT (B) was injected intravenously into SCID mice (n=3 for A and B). The serum content of human alpha 1-antitrypsin (hAAT) was determined by means of ELISA.

In this way, the proliferation-specific activity of the YB-1 promoter was proven.